Statistical Analysis Plan

A Phase 1, Open-label Study to Evaluate the Effects of NST-1024 on the Pharmacokinetics of Caffeine, Flurbiprofen, Omeprazole, Metoprolol, and Midazolam in Healthy Subjects

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LIST OF ABBREVIATIONS

Abbreviations pertain to the statistical analysis plan (SAP) only (not the tables, figures, and listings [TFLs]).

$\%AUC_{extrap}$	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity
ADaM	Analysis Data Model
AE	adverse event
$AUC_{0-\infty}$	area under the concentration-time curve from time 0 extrapolated to infinity
$AUC_{0-tlast}$	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
BLQ	below the limit of quantification
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL/F	apparent total clearance
C_{max}	maximum observed concentration
COVID-19	coronavirus disease 2019
CSR	clinical study report
CV	coefficient of variation
DMP	data management plan
ECG	electrocardiogram
eCRF	electronic case report form
GLSM	geometric least squares mean
ICF	informed consent form
ICH	International Council for/Conference on Harmonisation
LLOQ	lower limit of quantification
ln	natural log
LSM	least squares mean
MedDRA	Medical Dictionary for Regulatory Activities
MR_{AUC}	metabolite:parent ratio based on $AUC_{0-\infty}$
PK	pharmacokinetic(s)
QD	once daily
R^2-adj	adjusted coefficient for determination of exponential fit
SAP	statistical analysis plan
SD	standard deviation
SDV	source document verification
$t_{1/2}$	apparent terminal elimination half-life
TEAE	treatment-emergent adverse event

TFL	table, figure, and listing
t_{last}	time of the last quantifiable concentration
t_{max}	time of the maximum observed concentration
V_z/F	apparent volume of distribution during the terminal phase
WHODrug	World Health Organization Drug Dictionary
λ_z	apparent terminal elimination rate constant
λ_z Lower	start of exponential fit
λ_z N	number of data points included in the log-linear regression
λ_z Span Ratio	time period over which λ_z was determined as a ratio of $t_{1/2}$
λ_z Upper	end of exponential fit

1. INTRODUCTION

This SAP has been developed after review of the clinical study protocol (Final Version 1 dated 16 May 2022) and electronic case report form (eCRF).

This SAP describes the planned analysis of the pharmacokinetic (PK), safety, and tolerability data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shells document.

In general, the analyses are based on information from the protocol, unless they have been modified by agreement with NorthSea Therapeutics B.V. A limited amount of information about this study (eg, objectives, study design) is given to help the reader's interpretation.

This SAP must be finalized prior to the lock of the clinical database. Additionally, the SAP and TFL shells should be finalized prior to any programming activities commencing.

This SAP supersedes any statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified accordingly in the CSR. Any substantial deviations from this SAP will be agreed with NorthSea Therapeutics B.V. and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E3 guideline *Structure and Content of Clinical Study Reports*, ICH E8 guideline *General Considerations for Clinical Trials*, and ICH E9 guideline *Statistical Principles for Clinical Trials*.[1,2,3]

The document history is presented in Appendix 1.

2. STUDY OBJECTIVES AND ENDPOINTS

Table 1 shows the objectives and endpoints of the study.

Table 1: Objectives and Endpoints

Objectives	Endpoints
Primary:	
• to evaluate the effects of NST-1024 on the PK of caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam in healthy male and female subjects	• primary PK parameters: $AUC_{0\text{-inf}}$, $AUC_{0\text{-tlast}}$, and C_{max}
Secondary:	
• to assess the safety and tolerability of NST-1024 in healthy subjects	• incidence and severity of AEs • incidence of laboratory abnormalities, based on haematology, clinical chemistry, and urinalysis test results • 12-lead ECG parameters • vital signs measurements • physical examinations

Abbreviations: AE = adverse event; $AUC_{0\text{-inf}}$ = area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-tlast}}$ = area under the concentration-time curve from time 0 to the time of the last quantifiable concentration; C_{max} = maximum observed concentration; ECG = electrocardiogram; PK = pharmacokinetic(s).

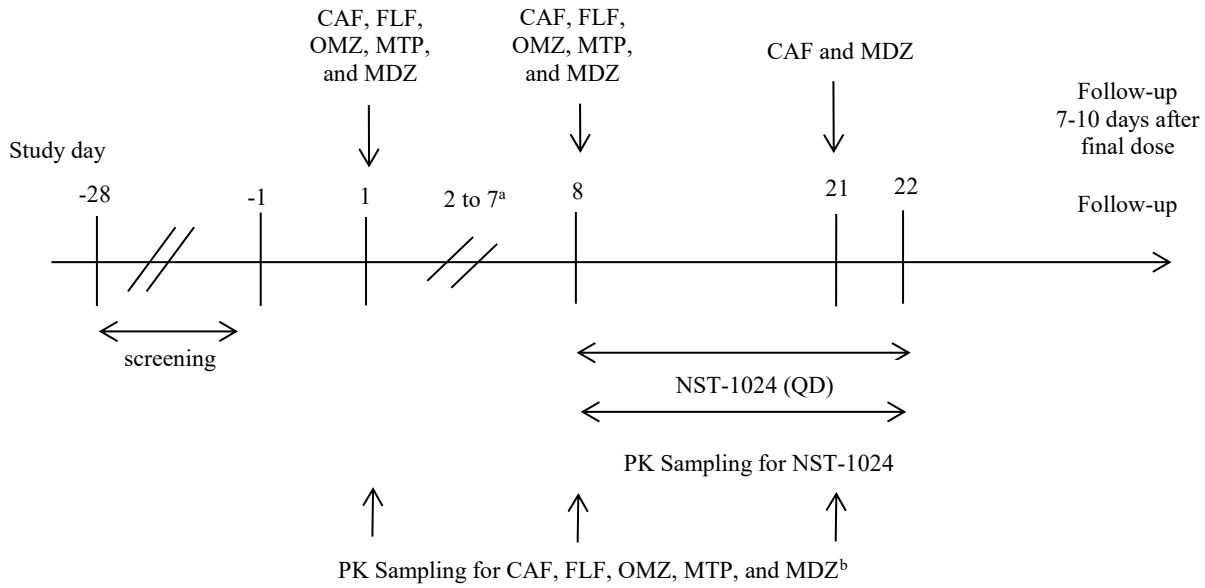
3. STUDY DESIGN

This will be a Phase 1, open-label study to evaluate the effects of NST-1024 on the PK of caffeine (and paraxanthine), flurbiprofen, omeprazole, metoprolol, and midazolam (and 1-hydroxymidazolam) in healthy male and female subjects. Up to 21 subjects will be enrolled to ensure that 18 subjects complete the study. All subjects will receive each of the following treatments:

- **Day 1:** single oral dose of 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam
- **Days 8 to 22:** oral doses of 200 mg NST-1024 once daily (QD) multiple-dose regimen
- **Day 8:** single oral dose of 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam coadministered with an oral dose of 200 mg NST-1024
- **Day 21:** single oral dose of 100 mg caffeine and 2.5 mg midazolam coadministered with an oral dose of 200 mg NST-1024.

An overview of the study design in Figure 1.

Figure 1: Study Schematic



Abbreviations: CAF = caffeine; FLF = flurbiprofen; MDZ = midazolam; MTP = metoprolol; OMZ = omeprazole; PK = pharmacokinetic(s); QD = once daily
[a] Subjects will discharge on Day 3 and return for check-in on Day 7.
[b] Pharmacokinetic sampling for CAF, FLF, MDZ, MTP, and OMZ on Days 1 and 8: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, and 48 hours postdose. Pharmacokinetic sampling for CAF and MDZ on Day 21: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, and 48 hours postdose.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Subjects will be admitted into the study site on Day -1 and be confined until discharge on Day 3. Subjects will return for check-in on Day 7 and be confined until discharge on Day 23. Subjects will return to the study site for a follow-up visit 7 to 10 days after the last dose.

The total duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 61 days.

The start of the study is defined as the date the first subject signs an informed consent form (ICF). The point of enrolment occurs at the time of subject number allocation. The end of the study is defined as the date of the last subject's last assessment (scheduled or unscheduled).

4. SAMPLE SIZE JUSTIFICATION

Up to 21 subjects will be enrolled in order that 18 complete the study.

5. STUDY TREATMENTS

The study treatment names and ordering to be used in the TFLs are presented in Table 2.

Table 2: Presentation of Study Treatments in TFLs

Study Treatment	Order in TFLs
Probe Drugs Cocktail#1	1
200 mg NST-1024 + Probe Drugs Cocktail#1	2
200 mg NST-1024 (QD)	3
200 mg NST-1024 (QD) + Probe Drugs Cocktail#2	4

QD = once daily
Probe drugs cocktail#1 = 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam; probe drugs cocktail#2 = 100 mg caffeine and 2.5 mg midazolam

The study treatment sequence names, abbreviations, and ordering to be used in the TFLs are presented in Table 3.

Table 3: Presentation of Study Treatment Sequence in TFLs

Study Treatment Sequence	Order in TFLs
Probe Drugs Cocktail#1 on Day 1; 200 mg NST-1024 + Probe Drugs Cocktail#1 on Day 8; 200 mg NST-1024 (QD) on Days 9 to 20; 200 mg NST-1024 (QD) + Probe Drugs Cocktail#2 on Day 21; 200 mg NST-1024 (QD) on Day 22	1

QD = once daily
Probe drugs cocktail#1 = 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam; probe drugs cocktail#2 = 100 mg caffeine and 2.5 mg midazolam

The '(Days X to Y)' part of the treatment and treatment sequence labels will be kept unchanged even if a subject misses a dose on 1 or more days. Exact dosing regimen details including duration and days on which dose was received will be presented in the treatment administration listing only.

6. DEFINITIONS OF POPULATIONS

Any protocol deviations, including those due to coronavirus disease 2019 (COVID-19) and related restrictions (see Section 7.1.1), will be considered prior to database lock for their importance and taken into consideration when assigning subjects to populations.

6.1. All Subjects Population

The all subjects population will include all subjects who signed the ICF and had any study assessment recorded in the database per the protocol.

6.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study treatment (NST-1024, caffeine, flurbiprofen, omeprazole, metoprolol, or midazolam).

6.3. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of active study treatment (NST-1024, caffeine, flurbiprofen, omeprazole, metoprolol, or midazolam) and have at least 1 valid PK concentration.

7. STATISTICAL METHODOLOGY

7.1. General

Listings will be provided for all data captured in the database, with the exception of medical history. Listings will include all subjects assigned to the all subjects population and include data up to the point of study completion or discontinuation. Subjects are generally considered to have completed the study if they complete the scheduled follow-up visit (rather than early termination visit). Any subject who discontinues the study will be identified accordingly in the listings. Summaries and statistical analyses will include the subjects assigned to the relevant population based on data type.

Data analysis will be performed using the SAS® statistical software package Version 9.4 (or higher if a new version is issued during the study).

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 (or higher if a new version is issued during the study) and CDISC ADaM Implementation Guide Version 1.1 (or higher if a new version is issued during the study). Pinnacle 21 Community Validator Version 4.0.1 (or higher if a new version is issued during the study) will be utilized to ensure compliance with CDISC standards.

For all statistical analyses, the hypothesis testing will be 2-sided and carried out on 0.05 significance level, unless specifically stated otherwise.

Caution should be used when interpreting results from the statistical analyses conducted in this study because the sample size is not based on power calculations.

Where reference is made to ‘valid’ data, this refers to non-missing data which meet the predetermined criteria (eg, are not flagged for exclusion).

Where reference is made to ‘all calculations’, this includes, but is not limited to, summary statistics, statistical analyses, baseline derivation, and changes from baseline.

All figures will be produced on linear-linear or discrete-linear scales, as applicable, unless specifically stated otherwise.

7.1.1. Handling of Data Quality Issues Due to Coronavirus Disease 2019 and Related Restrictions

Due to COVID-19 and related restrictions, there is a high risk for impact to data integrity, with the recognized potential for:

- Missed visits, caused by, for example:
  - Subject unable to travel to site due to restrictions, the need to quarantine, or COVID-19 infection
  - Subject unwilling to go to site due to fear of COVID-19 infection
  - Site postponing subject's visit due to investigator not being available (eg, if they have been dispatched to hospital handling COVID-19 infections)
- Site unable to replenish supply of investigational product
- Incomplete data entry by sites due to limited resources to support study or no access to source documents or to eCRF
- Outstanding source document verification (SDV) due to sponsor or country restrictions on remote SDV, or no or limited access to site(s) for on-site visits
- Unanswered queries

At the time of the reporting of the study results, all protocol deviations due to COVID-19 or related restriction will be assessed for their severity and impact on the analyses. If needed, appropriate statistical methods will be applied as a mitigating action (eg, data might be categorized into 2 analysis groups, with and without COVID-19 and related restrictions impact); however, this will exclude any imputations of the missing values. Any mitigating actions will be agreed with NorthSea Therapeutics B.V. in advance and identified in the CSR.

7.1.2. Calculation of the Summary Statistics

For continuous data the following rules will be applied:

- Missing values will not be imputed, unless specifically stated otherwise.
- Unrounded data will be used in the calculation of summary statistics.
- If the number of subjects with valid observations (n) <3, summary statistics will not be calculated, with the exception of n, minimum, and maximum.
- In general, as early termination data are not associated with any scheduled timepoint, they will be excluded from all calculations of summary statistics. Exceptions may be made where justified.

For categorical data the following rules will be applied:

- For ordered categorical data (eg, adverse event [AE] severity), all categories between the possible minimum and maximum categories will be included, even if n = 0 for a given category.

- For non-ordered categorical data (eg, race), only those categories for which there is at least 1 subject represented will be included; unless specifically stated otherwise.
- Missing values will not be imputed, unless specifically stated otherwise. A 'missing' category will be included for any parameter for which information is missing. This will ensure that the population size totals are consistent across different parameters.

7.1.3. Triplicate Readings

For vital signs data only, where triplicate readings are taken, the median of triplicate readings will replace the separate individual triplicate readings in all calculations.

For 12-lead electrocardiogram (ECG) data only, where triplicate readings are taken, the mean of triplicate readings will replace the separate individual triplicate readings in all calculations.

In case of incomplete triplicate readings (eg, only 2 out of 3 readings were recorded), the mean and/or medians will be calculated, as appropriate, based on the number of readings available.

7.1.4. Repeat and Unscheduled Readings

For vital signs and 12-lead ECG data only, any predose value recorded in addition to the original value or a postdose value recorded within 15 minutes of the original value will be defined as a repeat value; any postdose value recorded more than 15 minutes after the original value will be defined as an unscheduled value. For all other data types (eg, laboratory parameters), any value recorded in addition to the original value will be defined as an unscheduled value.

The original value will be replaced by the last associated repeat value in all calculations.

As unscheduled values are not associated with any scheduled timepoint, they will be excluded from all calculations, with the exception of the baseline derivation (see Section 7.1.5).

7.1.5. Definitions of Baseline and Change from Baseline

The baseline will be defined as the last value recorded prior to the first dose. If the date/time of the value is incomplete or missing, it will be excluded from the baseline calculation, unless the incomplete date/time indicates the value was recorded prior to the first dose.

Individual changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the postdose timepoint.

The summary statistics for change from baseline will be derived from individual subjects' values (eg, mean change from baseline will be the mean of the individual changes from baseline for all subjects, rather than difference between the mean value at the postdose timepoint and mean value at baseline).

See Section 7.1.4 for more detail on handling repeat and unscheduled readings in the calculations. See Section 7.1.3 for more detail on handling of triplicate readings in the calculations.

7.2. Subject Disposition and Population Assignment

Subject disposition and population assignment will be listed.

A summary table by overall will be provided, based on the safety population.

7.3. Screening Demographics

The screening demographics including age, sex, race, ethnicity, height, body weight, and body mass index will be listed.

A summary table by overall will be provided, based on the safety population.

7.4. Prior and Concomitant Medication

Prior medication will be defined as medication that ends prior to the first dose. Concomitant medication will be defined as medication that starts during or after the first dose or starts but does not end prior to the first dose.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) Global, Format B3, Version March 2022 (or later if a new version is issued during the study; see the data management plan [DMP] for more details). Prior and concomitant medications will be listed.

7.5. Pharmacokinetic Assessments

7.5.1. Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma concentrations of flurbiprofen, omeprazole, and metoprolol on profile days 1 and 8, and for caffeine (and metabolite paraxanthine) and midazolam (and metabolite 1-hydroxymidazolam) on profile days 1, 8, and 21 using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1 or higher):

Parameter	Units[a]	Definition
$AUC_{0\text{-}tlast}$	h*ng/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last})[b]
$AUC_{0\text{-}\infty}$	h*ng/mL	area under the concentration-time curve from time 0 extrapolated to infinity[c]
$\%AUC_{extrap}$	%	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration

$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance (parent analyte only)
V_z/F	L	apparent volume of distribution during the terminal phase (parent analyte only)
MR_{AUC}		metabolite:parent ratio based on $AUC_{0-\infty}$ (metabolites only)

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
[c] Based on the last observed quantifiable concentration

Additional PK parameters may be determined where appropriate.

Pharmacokinetic analysis will be carried out where possible using actual dose administered (mg) and actual postdose blood sampling times. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

The parameters C_{max}, t_{last}, and t_{max} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 timepoint, t_{max} will be assigned to the first occurrence of C_{max}.

The metabolite:parent ratio (MR_{AUC}) will be calculated as follows:

MR_{AUC} = ($AUC_{0-\infty}$ metabolite / MW metabolite) / ($AUC_{0-\infty}$ parent drug / MW parent drug), where MW is the molecular weight of each analyte.

The molecular weights of parent analyte and metabolites to be used in the adjustment are as follows:

caffeine = 194.19 g/mol

paraxanthine = 180.16 g/mol

midazolam = 325.78 g/mol

1-hydroxymidazolam = 341.8 g/mol

The parameter $AUC_{0-tlast}$ or other common partial area may be used to determine MR_{AUC} if $AUC_{0-\infty}$ cannot be reliably calculated for the majority of subjects.

7.5.1.1. Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7. Parameters requiring λ_z for their calculation (eg, $AUC_{0-\infty}$, $t_{1/2}$, CL/F, V_z/F, and V_{ss}/F) will only be calculated if the R^2-adj value of the regression line is ≥0.7.

The following regression-related diagnostic PK parameters will be determined, when possible:

Parameter	Units	Definition
λ_z	1/h	apparent terminal elimination rate constant
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N	NA	number of data points included in the log-linear regression
λ_z Span Ratio	NA	time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj	NA	adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (ie, the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2, the robustness of the $t_{1/2}$ values will be discussed in the clinical study report (CSR).

7.5.1.2. Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of AUC will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow C_{max}. An exception may be made for metabolites, where C_{max} may be the last timepoint.

If the extrapolated area is >30%, $AUC_{0-\infty}$ (and derived parameters) may be excluded from the summary statistics and statistical analysis at the discretion of the sponsor or pharmacokineticist and any such occurrences will be identified in the report.

If $AUC_{0-\infty}$ cannot be determined reliably for all subjects and/or treatments, an alternative AUC measure, such as AUC to a fixed timepoint or $AUC_{0-tlast}$, may be used in the statistical analyses.

7.5.1.3. Criteria for Handling Concentration Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQ concentrations will be treated as missing. The following rules apply to the specific situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.

- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.

- If a predose plasma concentration is missing, it will be set to 0 by default within Phoenix WinNonlin.

7.5.1.4. Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data must have strong justification and will be documented in the CSR.

Any quantifiable predose concentration value in the first treatment period will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default in Phoenix WinNonlin.

If the predose concentration is >5% of C_{max} in the second or any subsequent treatment period, all PK concentration and parameter data will be excluded from the summary statistics and statistical analysis for that period.

7.5.2. Presentation of Pharmacokinetic Data

All PK concentrations and parameters will be listed.

Summary tables, arithmetic mean (+ standard deviation [SD]) figures, overlaying individual figures, and individual figures by treatment and time postdose will be provided for plasma PK concentrations. All figures will be produced on both linear-linear and linear-logarithmic scales, with the exception of figures across all days, which will be produced on the linear-linear scale only. The +SD bars will only be displayed on the linear-linear scale.

Summary tables by treatment will be provided for all PK parameters, with the exception of diagnostic regression-related PK parameters.

A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median t_{max}.

If the actual time of sample collection deviates from the nominal time by more than ±10%, the concentration will be flagged in the listings.

Individual concentrations deemed to be anomalous will be flagged in the listings and excluded from the summary statistics.

For plasma concentration data the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics.

- Arithmetic mean or median values that are BLQ will be presented as 0.

For PK parameters the following rule will apply:

- Geometric mean and coefficient of variation will not be calculated for t_{last} or t_{max}.

7.5.3. Pharmacokinetic Statistical Methodology (Drug-drug Interaction)

A statistical analysis will be conducted to investigate the drug-drug interaction of NST-1024 on the PK of the probe drugs by comparing:

- 200 mg NST-1024 + Probe Drugs Cocktail#1 (test treatment) to Probe Drugs Cocktail#1 (reference treatment). This comparison will be done for caffeine, paraxanthine, flurbiprofen, omeprazole, metoprolol, midazolam, and 1-hydroxymidazolam analytes separately.

- 200 mg NST-1024 (QD) + Probe Drugs Cocktail#2 (test treatment) to Probe Drugs Cocktail#1 (reference treatment). This comparison will be done for caffeine, paraxanthine, midazolam, and 1-hydroxymidazolam analytes separately.

The natural log (ln)-transformed[5] $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} will be analyzed using a mixed model.[6] The model will include actual treatment as fixed effect and subject as a random effect.

For each PK parameter separately, the least squares mean (LSM) for each treatment, difference in LSMs between the test and reference treatments, and corresponding 90% confidence interval (CI) will be calculated; these values will then be back-transformed to give the geometric least square mean (GLSM), ratio of GLSMs, and corresponding 90% CI.

Additionally, the pooled estimate (across treatments) of the within-subject coefficient of variation (CV) will be calculated, and residual plots will be produced to assess the adequacy of the model(s) fitted.

Examples of the SAS code that will be used are as follows:

Mixed Model Analysis

```
proc mixed data = <data in>;
  by parcat1n parcat1 pkday paramn param;
  class trtan usubjid;
  model lpk = trtan / cl residual ddfm = kr2;
  lsmeans trtan / cl pdiff = control('1') alpha = 0.1;
  random intercept / subject = usubjid;
  ods output lsmeans = <data out>;
  ods output diffs = <data out>;
  ods output covparms = <data out>;
```

```
run;
```

7.6. Safety and Tolerability Assessments

7.6.1. Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 25.0 (or higher if a new version is issued during the study; see the DMP for more details).

A treatment-emergent adverse event (TEAE) will be defined as an AE that starts during or after the first dose, or starts prior to the first dose and increases in severity after the first dose.

A treatment-related TEAE will be defined as a TEAE with a relationship of possibly related or related to the study treatment, as determined by the investigator.

The assignment of TEAEs to treatments will be as follows:

- A TEAE occurring during or after Day 1 dosing and prior to Day 8 dosing will be assigned to 'Probe Drugs Cocktail#1'.
- A TEAE occurring during or after Day 8 dosing and prior to Day 10 dosing will be assigned to '200 mg NST-1024 + Probe Drugs Cocktail#1'.
- A TEAE occurring during or after Day 10 dosing and prior to Day 21 dosing will be assigned to '200 mg NST-1024 (QD)'.
- A TEAE occurring during or after Day 21 dosing will be assigned to '200 mg NST-1024 (QD) + Probe Drugs Cocktail#2'.

All AEs will be listed. In addition to the data recorded in the database, the listings will include derived onset time and duration. Onset time will be calculated from the time of the last associated dose for TEAEs only. Where the last associated dose is referring to the last dose received prior to the start of a TEAE.

The frequency of subjects with TEAEs and the number of TEAEs will be summarized for the following categories:

- TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- TEAEs by severity and treatment
- Treatment-related TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- Treatment-related TEAEs by severity and treatment

The frequency of subjects will be summarized separately for TEAEs and treatment-related TEAEs by the following:

- System organ class, preferred term, and treatment
- Preferred term and treatment

For the AE data the following rules will apply:

- For the derivation of treatment-emergent status (applicable to all AEs): If the start date/time of an AE is incomplete or missing, an AE will be assumed to not be a TEAE, unless the incomplete start date/time or the end date/time indicates an AE started after the first dose.
- For the derivation of treatment-related status (applicable to TEAEs only): If the study treatment relationship for a TEAE is missing, a TEAE will be assumed to not be a treatment-related TEAE.
- For the derivation of onset time (applicable to TEAEs only): If the start date/time of a TEAE is missing, onset time will not be calculated. If the start date/time of a TEAE is incomplete, where possible, the minimum possible onset time will be calculated and presented in '≥DD:HH:MM' format (eg, if the date/time of the last associated dose is 01MAY2019/08:00 and recorded start date/time of a TEAE is 03MAY2019, then the minimum possible onset time will be calculated by assuming a TEAE started at the first hour and minute of 03MAY2019 [03MAY2019/00:00], thus will be presented as onset time ≥01:16:00 in the listing). If the start date of a TEAE is the same as the date of the last associated dose but the start time of a TEAE is missing, an onset time will be presented as '≥00:00:01'. Any clock changes will be accounted for in the derivation.
- For the derivation of duration (applicable to all AEs): If the end date/time of an AE is missing, duration will not be calculated. If the start or end date/time of an AE is incomplete, where possible, the maximum possible duration will be calculated and presented in '≤DD:HH:MM' format (eg, if the start of an AE date/time is 01MAY2019/08:00 and its recorded end date/time is 03MAY2019, then the maximum possible duration will be calculated by assuming an AE ended at the last hour and minute of 03MAY2019 [03MAY2019/23:59], thus will be presented as duration ≤02:15:59 in the listing). Any clock changes will be accounted for in the derivation.
- For the calculation of TEAE summary statistics: If the severity of a TEAE is missing, that TEAE will be counted under the 'missing' category.
- For the calculation of TEAE summary statistics: If a subject experienced multiple TEAEs with the same preferred term for the same treatment, this will be counted as 1 TEAE for that treatment under the maximum severity recorded.

7.6.2. Clinical Laboratory Parameters

All clinical laboratory parameters will be listed; any value outside the clinical reference range will be flagged. Separate listings will be provided for any parameter for which there is any individual subject value outside the respective clinical reference range.

Summary tables and boxplots by timepoint will be provided for clinical chemistry and hematology parameters.

Values recorded as <x, ≤x, >x, or ≥x will be displayed in the listings as recorded. For the derivation of listing flags, all calculations, and presentation in the figures, <x and ≤x values will be set to half of x, whereas >x and ≥x values will be set to x.

7.6.3. Vital Signs Parameters

All vital signs parameters and their changes from baseline will be listed, as applicable; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by timepoint will be provided for all vital signs parameters and their changes from baseline.

7.6.4. 12-lead Electrocardiogram Parameters

All 12-lead ECG parameters and their changes from baseline will be listed; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by timepoint will be provided for all 12-lead ECG parameters and their changes from baseline.

7.6.5. Other Assessments

Medical history will not be listed.

All other safety and tolerability assessments not detailed in the above sections will be listed only.

7.6.6. Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

8. INTERIM ANALYSES

No interim analyses are planned for this study.

9. SIGNIFICANT CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSES

There were no significant changes from the protocol-specified analyses.

10. REFERENCES

1. ICH. ICH Harmonised Tripartite Guideline: Structure and content of clinical study reports (E3). 30 November 1995.

2. ICH. ICH Harmonised Tripartite Guideline: General considerations for clinical trials (E8). 17 July 1997.

3. ICH. ICH Harmonised Tripartite Guideline: Statistical principles for clinical trials (E9). 5 February 1998.

5. Keene ON. The log transformation is special. *Stat Med*. 1995;14(8):811-819.

6. Brown H, Prescott R. *Applied Mixed Models in Medicine*. Chichester: John Wiley & Sons, 1999.

11. APPENDICES

Appendix 1: Document History

Status and Version	Date of Change	Summary/Reason for Changes
Final Version 1.0	NA	NA; the first version.

NA = not applicable

Statistical Analysis Plan (SAP)/Initiation of Programming Approval Form

Type of Approval (select one) : ☒ **SAP** ☐ **Initiation of Programming**

Sponsor Name:	NorthSea Therapeutics B.V.		
Sponsor Protocol ID:	NST-1024-02	**Labcorp Study ID:**	8489933
SAP text filename:	NST-1024-02_SAP_Final_V1.0.pdf		
TFL shells filename:	NST-1024-02_TFL_Shells_Final_V1.0.pdf		
Version:	1.0	**Date:**	05OCT2022

Labcorp Approval(s):

Lead Statistician

Approval Signature Print Name Date	DocuSigned by: Izabela Antys Signer Name: Izabela Antys Signing Reason: I approve this document Signing Time: 05 Oct 2022 | 6:49:07 PM EDT CB7E74340A8D4D14A0CC533E6B3BB398

Sponsor Approval(s):

By signing below when the statistical analysis plan (SAP) is considered final, the signatories agree to the analyses to be performed for this study and to the format of the associated tables, figures, and listings (TFLs). Once the SAP has been signed, programming of the Analysis Dataset Model (ADaM) datasets and TFLs based on these documents can proceed. Any modifications to the SAP text and TFL shells made after signing may result in a work-scope change.

Approval Signature Print Name Date	DocuSigned by: Michelle Yokley Signer Name: Michelle Yokley Signing Reason: I approve this document Signing Time: 06 Oct 2022 | 1:25:21 AM CEST 9D8A3D5812C34B2897D7A14001BB4409

16.1.9.2. Quality Tolerance Limit Definitions

Parameter	Justification for Parameter	Unit Tolerance
Minimum number of evaluable subjects needed.	A shortfall in the overall number of subjects could have had a significant impact on interpretation of the primary endpoint because of limited/insufficient exposure.	N: 21